IS THE ERYTHROCYTE PERMEABLE TO HYDROGEN IONS?

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I

The erythrocyte is generally believed to differ from most other cells in the ease with which its internal reaction is influenced by that of its surroundings. Even in the absence of so-called "penetrating" acids and bases such as CO_2, fatty acids, ammonia, etc., which alone are effective with ordinary cells, it is easy to bring about in it striking internal pH changes. The mere hydrogen ion concentration (strictly speaking, the hydrogen ion activity) of the surrounding medium, regardless of how it is produced, seems automatically to determine in the erythrocyte an internal hydrogen ion concentration that can be predicted by the principles of the Donnan equilibrium (Warburg, 1922; Van Slyke, Wu and McLean, 1923). This interrelation of external and internal pH is part of an important mechanism for preserving an approximately constant blood reaction under all ordinary physiological conditions.

While the establishment of a Donnan equilibrium between the erythrocyte and its surroundings is almost universally considered to be due to a passage of ions across the cell membrane, there has been some doubt as to the particular ions involved in the case of pH changes. Partly, no doubt, owing to our customary methods of measuring and defining the reactions of aqueous solutions, and partly to the fact that physiologists, in general, have had to deal more frequently with the penetration of cells by acids than by alkalies, it has been customary in the past to postulate a ready permeability of the erythrocyte to hydrogen ions. But it has been pointed out by Van Slyke, Wu and McLean (1923) and others that exactly the same end results would be obtained if the permeability were to hydroxyl rather than to hydrogen ions, since, as is well known, the relation existing between these two ions in aqueous solutions is such that the activity of the one is related in a fixed manner to that of the other.

In cases where our interest is primarily in equilibria, it is a matter of indifference whether the cell is permeable to the hydrogen or to the hydroxyl ion, or to both; indeed, by the study of equilibrium

states alone it is impossible to reach any decision as to the mechanism by which these states have been reached. Since most of the work heretofore done on the erythrocyte has been concerned with equilibria, it is not surprising that the question of the relative penetrating powers of hydrogen and hydroxyl ions has received almost no attention. However, this question is obviously one of considerable importance to those interested primarily in the mechanism of cell permeability. The erythrocyte, as is well-known, appears to be permeable to anions and impermeable to cations (for a summary of the evidence see Jacobs, 1931), and a single exception to the general rule would make necessary a more complicated theory of ionic permeability than would otherwise be required. It is true that the hydrogen ion is unique in several other respects, and it is by no means inconceivable that it might be in its powers of penetrating the erythrocyte as well. It would, however, considerably simplify the situation if direct evidence could be furnished in favor of the other alternative which is, *a priori*, the more probable of the two.

In the present paper evidence of this sort has been obtained by the use of a simple method, which may, incidentally, prove to be useful in the investigation of other problems connected with the physiology of the erythrocyte. Though this evidence is not presented as conclusive proof of the view that pH adjustments between the erythrocyte and its surroundings are produced through the agency of the hydroxyl ion, it is believed that the observed facts may be explained more simply in this way than in any other; indeed, we have been unable to find any other simple and plausible explanation that even remotely fits the facts.

II

One of the most convenient methods for studying permeability to hydrogen and hydroxyl ions is to employ cells which contain natural indicators of some sort whose color is affected by changes in intracellular reaction. It happens that the erythrocyte belongs in the category of cells showing such color changes. As is well known, hemoglobin, in the presence of sufficiently high concentrations of acids, loses its red color and becomes converted into brownish, or in dilute solutions, yellowish acid hematin. The fact that this change is irreversible and is not associated with a definite pH value prevents it from being employed in exactly the same way as those of true indicators; but it may, nevertheless, when used with judgment, be of considerable usefulness. In the case of hemolyzed blood in which intimate contact of the acid with the hemoglobin is immediately insured, the color change in the presence of 0.15 M NaCl occurs

practically instantly at pH values of 2.5 or lower; at pH 3.5 the time required may be several minutes and at pH 4.0 may be measured in hours. As far as can be determined by the rather crude methods available, there is no sharp upper limit at which the change entirely ceases.

The same color change occurs, but much more slowly, in the case of suspensions of unhemolyzed erythrocytes; and it is this fact that renders it useful as an indicator of the penetration of acid into the cells. Since, unfortunately, it does not take place sharply at any fixed pH value, it is not a highly accurate means of measuring acid penetration; but within certain limits it appears to be capable of being employed as at least an approximate measure of the rate of this process. The color changes with intact erythrocytes are naturally most conspicuous when the cells are sufficiently numerous to give a distinctly red color to the suspension. A mixture of 1 part of blood to 250–500 of solution (1 or 2 drops to 25 cc.) is that which we have found most suitable; with greater dilutions the color of the suspension is yellowish to begin with and the color change consequently less distinct; with higher concentrations of blood the effect on the pH of the solutions is too great. The color change may be made considerably more striking by first treating the erythrocyte suspension with a trace of carbon monoxide, but to avoid the possibility of unknown complications this procedure was not employed in the present experiments.

Under most conditions which bring about a change in the color of the cells, hemolysis also occurs. The question therefore arises whether the acid penetrates the cells and acts upon the hemoglobin within them or whether the reaction is rather an extracellular one with hemoglobin first liberated by hemolysis. We believe that the former alternative is probably the correct one, for two reasons. In the first place, careful observations have been made, by the method of Jacobs (1930*b*), of the time at which the first traces of hemolysis appear. This proves almost invariably to be after rather than before a distinct color change has occurred (see Tables I, II and III). In the second place, it has been found that within a certain pH range the presence of Na_2SO_4 in proper amounts may either prevent hemolysis almost completely or at least delay it for an hour or more without at all slowing the change of color. Indeed, by starting with a 10 per cent saccharose solution containing 0.015 N HCl, the addition of Na_2SO_4 to a concentration of 0.05 M may considerably accelerate the color change, while strongly retarding hemolysis. In one experiment the following figures were obtained:

	Color Change	Hemolysis
Saccharose alone	285 seconds	390 seconds
Saccharose + Na_2SO_4	165 seconds	>1 hour

The probable reason for the accelerating effect of Na_2SO_4 on the color change will be discussed below. That for the inhibition of hemolysis is not certainly known, but at all events such experiments furnish a clear demonstration of the possibility of intracellular color changes.

Though liberation of hemoglobin from the cells is not necessary in order that the reaction may occur, it must not be thought that the entrance of acid is not influenced by injury to the cells. In observing the behavior of a given suspension, it may be noted that the color change does not proceed gradually and fairly regularly, as it does in the case of hemolyzed blood at sufficiently high pH values, but that for a time the change is extremely slow or entirely invisible, and then suddenly becomes much more rapid, as if a barrier of some sort had been broken down by injury to the cell. Shortly after this point, hemolysis occurs. As contrasted with the slow rate at which the color change is produced by HCl, that in the presence of so-called "penetrating" acids, such as acetic or butyric acids at the same or even considerably less acid pH values, is strikingly rapid. In this respect the behavior of the erythrocyte is similar to that of ordinary cells containing natural or artificially introduced indicators.

In the experiments here reported the erythrocytes were obtained from defibrinated ox blood. The cells were not "washed," since it was believed to be less serious to introduce into the solutions employed the slight traces of blood proteins unavoidably present in dilutions of 1 : 250 or 1 : 500 than perhaps to change the fundamental properties of the erythrocytes in the manner described by Kerr (1929) by previously removing these proteins. The pH determinations were in all cases made with the quinhydrone electrode at the conclusion of each set of experiments. Though the addition of the blood somewhat reduced the acidity of the original solutions, the latter were present in such excess that the pH changes in most cases did not amount to more than a few tenths, or at the highest concentrations of acid, a few hundredths of a pH unit.

The color changes were determined in test tubes by eye, the time given being that at which a distinct color difference could be detected between the experimental tube and an appropriate control. Though it was found that a certain degree of refinement could be introduced by the use of a colorimeter, the advantages of this instrument for the present purposes were not sufficient to compensate for the considerably

greater time required with it to carry out a series of experiments. The determinations of the time of hemolysis, as has already been mentioned, were made by the method of Jacobs (1930*b*), of necessity on a separate, but as nearly as possible identical, suspension of the same blood in the same solution. The few cases in which hemolysis appeared slightly to precede the color change are probably to be accounted for by the fact that the figures were obtained not from a single experiment but from two parallel experiments.

III

The method described in the preceding section was first employed by us in an attempt to throw further light upon an earlier observation (Jacobs, 1930*a*) that the rate of acid hemolysis is greatly influenced by the salt concentration of the external medium. Illustrations of

TABLE I

Effect on time of color change and of hemolysis of adding different amounts of NaCl to 0.02 M HCl in 0.3 M saccharose. All times are in seconds.

Concentration of NaCl	Color change	Beginning of hemolysis	75 per cent hemolysis
—	116	130	153
0.0002	118	127	147
0.0004	108	128	135
0.0008	104	—	—
0.0016	110	—	—
0.0031	116	—	—
0.0063	111	123	137
0.0125	102	111	125
0.025	87	95	119
0.05	71	80	92
0.1	60	71	81
0.2	45	72	80

this effect will be found in Table I and in Tables II and III which, taken together, show the same thing.

The more rapid rate of acid hemolysis in the presence of NaCl might conceivably be due to a more ready penetration of the cells by the acid; on the other hand, since hemolysis is a complicated process, it is also possible that the salt might accelerate the destruction of the erythrocyte in some other way. It was in an attempt to decide between these two possibilities that advantage was taken of a method by which the penetration of the acid could be made directly visible. It was thought that in the presence of different concentrations of salt, a parallel between the time of color change and of hemolysis, with the

color change preceding hemolysis, would indicate an effect of the salt on the actual rate of penetration of the acid. That such a parallel does in fact exist is shown by the experiments to which reference has already been made. In Table I, for example, it will be noted that concentrations of NaCl less than about 0.01 M have little or no effect on either process, but that at higher concentrations the acceleration of hemolysis very closely follows that of the color change.

The question now arises why the entrance of acid and the onset of hemolysis occur more quickly in the presence of NaCl than in its absence. Several conceivable explanations may immediately be dismissed as being decidedly improbable. For example, the case

TABLE II

Time (in seconds except where otherwise indicated) of Color Change and of Hemolysis with Different Concentrations of HCl in 0.15 M NaCl

Concentration of HCl	Color change (hemolyzed blood)	Color change (unhemolyzed blood)	Beginning of hemolysis	75 per cent hemolysis	pH after hemolysis
0.08 N	Almost instantaneous	8	12	25	1.26
0.04	Almost instantaneous	25	29	35	1.55
0.02	Almost instantaneous	39	39	50	1.95
0.016	Almost instantaneous	42	33	60	1.95
0.008	Almost instantaneous	46	44	90	2.25
0.004	Almost instantaneous	53	60	145	2.61
0.002	3	57	72	194	3.06
0.001	9	72	75	232	3.78
0.0005	135	480	67(?)	232	4.62
0.0001	3–4 hours	∞	∞	∞	—

presents superficial analogies with the increased permeability of various cells in the presence of NaCl (Harvey, 1911; Osterhout, 1911, 1922, etc.); but it is easy to show that the accelerated entrance of acid into the erythrocyte and the subsequent hemolysis occur equally readily in the presence of pure isotonic $CaCl_2$ or of physiologically balanced mixtures of NaCl and $CaCl_2$. It has, in fact, been found that a considerable variety of salts are, without exception, effective in facilitating the entrance of HCl into the erythrocyte, though, as mentioned above, sulfates in certain concentrations may inhibit the subsequent hemolysis. It is very improbable, therefore, that there is any close connection between this phenomenon and the older

observations on the increase of permeability produced by sodium salts, which is a specific effect peculiar to these and perhaps a few other salts, and which is readily antagonized by calcium.

Another explanation, applying specifically to the erythrocyte, was next considered, namely, that in the presence of salts some constituent of the cell surface might be removed, thus rendering the cell more permeable to ions. In this connection reference may be made to the work of Brinkman and van Dam (1920), who have reported that it is easy to remove lecithin from the erythrocyte by solutions of electrolytes but not by those of non-electrolytes. An explanation of this sort was, however, rendered very improbable by a simple experiment

TABLE III

Time (in seconds except where otherwise indicated) of Color Change and of Hemolysis with Different Concentrations of HCl in 0.3 M Saccharose

Concentration of HCl	Color change (hemolyzed blood)	Color change (unhemolyzed blood)	Beginning of hemolysis	75 per cent hemolysis	pH after hemolysis
0.32 N	Almost instantly	12	31	52	0.82
0.16	Almost instantly	15	24	29	1.03
0.08	Almost instantly	25	30	36	1.29
0.04	Almost instantly	56	59	69	1.50
0.02	Almost instantly	90	132	144	1.70
0.01	Almost instantly	185	231	269	2.04
0.005	3	270	330	450	2.26
0.0025	75	720	960	—	2.56
0.0012	240	3 hours	4 hours	—	2.87
0.0006	2 hours	5 hours	—	—	3.30

which consisted in comparing the effectiveness of previous washings of the cells with isotonic NaCl solutions, with the actual presence of small quantities of the salt at the time of hemolysis. In one such experiment it was found that ox erythrocytes were no more readily hemolyzed in 0.3 M glycerol containing 0.015 N HCl after six previous washings in isotonic NaCl than before. If the observed effect were merely due to the removal of something from the cell surface, six washings ought to have been far more effective than was the presence in the solution at the time of hemolysis of as low a concentration of NaCl as 0.01 M or less; but this was not the case. From this and similar experiments the conclusion was drawn that it is necessary for

the salt actually to be present with the HCl in order to exert its characteristic influence.

In an attempt to throw further light upon this question, systematic observations were made over a considerable range of acid concentrations both in the presence of NaCl (0.154 M) and in its absence, using for the latter purpose a 0.3 M solution of saccharose. Two such experiments, made on the same blood under as nearly as possible comparable conditions (except for a slight accidental difference, not believed to be significant, in the concentration of the blood in a few of the individual experiments) are summarized in Tables II and III.

The data contained in Tables II and III, which were obtained before the subject had received any theoretical treatment, seemed at first sight somewhat puzzling; but, as will be shown, they have proved to be capable of a very simple semi-quantitative explanation on the basis of a hypothetical permeability of the erythrocyte to hydroxyl rather than to hydrogen ions. It will be noted that in the experiments in question, as in previous ones, color changes and hemolysis always occurred more rapidly in the presence than in the absence of NaCl, other conditions being the same. But not only were the rates of color change and of hemolysis slower in solutions of saccharose than in those of NaCl but in the absence of salt both of these processes entirely failed to occur at concentrations of acid that were otherwise always effective. In other words, it would appear that the salt, in addition to its other effects, influences the position of final equilibrium of the system.

Another difference between the experiments represented in Tables II and III, whose meaning was at first far from clear but which we now believe to be of considerable theoretical significance, is that whereas in the non-electrolyte solutions, over a fairly wide range, the rate of color change and of hemolysis is roughly proportional to the concentration of acid (*i.e.*, doubling the concentration of acid approximately halves the time required for the attainment of the chosen end-point), in the NaCl solutions the concentration of acid is of much less importance. Thus, a forty-fold change in concentration (from 0.001 N to 0.04 N) is seen in Table II to decrease the time required for the color change only from 72 to 25 seconds and for the beginning of hemolysis from 75 to 29 seconds.

In an attempt to account for these various observations, we turned to a consideration of the conditions governing ionic exchanges of various sorts between the erythrocyte and its surroundings. Not only has this treatment of the problem furnished a plausible and simple explanation for all the facts mentioned above, but it has, in

addition, apparently thrown some light upon the equally important question of the relative permeability of the erythrocyte to hydrogen and to hydroxyl ions.

IV

The passage of ions across a membrane can occur only in such a way that electrical neutrality is at all times preserved. Thus, Cl', an ion which is known to pass readily between the erythrocyte and its surroundings, might do so by being exchanged for another univalent anion such as HCO'_3 or OH' or, if the cell were permeable to $H^{\cdot}$ ions, it might cross the membrane in company with one of these cations. The absolute rate of movement of pairs of ions, either in the same or in opposite directions, is not known, but in any given case, by an obvious application of the mass law, the rate of total movement at a given instant ought to be proportional to the product of the concentrations (or activities) of the two members of the pair. When the final equilibrium is reached the total movements in the two directions must balance, giving, therefore, as the condition for equilibrium either:

$$[H]_{solution} \times [Cl]_{solution} = [H]_{cell} \times [Cl]_{cell}$$

or

$$[OH]_{cell} \times [Cl]_{solution} = [OH]_{solution} \times [Cl]_{cell}$$

as the case may be. These expressions are identical with those obtained by Donnan by more rigorous thermodynamic reasoning.

In the case under consideration, it is unfortunately impossible to follow the entire course of the diffusion process by which the final theoretical ionic equilibrium tends to be established. Indeed, in a case where a cell is being hemolyzed and its hemoglobin is at the same time undergoing a fundamental chemical change, anything like an equilibrium is unthinkable. The only part of the process, therefore, about which we can form a reasonably accurate conception is its initial stage when the cell and the surrounding medium both have known compositions. Though information limited merely to this stage is less than we might desire, it is better than none at all; and, indeed, it seems reasonable to suppose that the conditions that determine the initial rate of ionic exchange would exert a similar influence over much of the remainder of the process. It will therefore be instructive to calculate the values of the initial mass law effect for several different types of solutions.

In Fig. 1 are represented the concentrations (which will here be employed as an approximation in place of the more accurate activities, and which are expressed in mols per kilo of water) of certain ions at the instant when a normal erythrocyte is placed in a given solution

FIG. 1

Cell	Solution
(8×10^{-2}) Hb'	
(16×10^{-2}) $K^{\cdot}$	$Na^{\cdot}$ (y)
(8×10^{-2}) Cl'	$Cl'\left(x+y-\frac{10^{-14}}{x}\right)$
(5×10^{-8}) $H^{\cdot}$	$H^{\cdot}$ (x)
(2×10^{-7}) OH'	$OH'\left(\frac{10^{-14}}{x}\right)$

containing as its only salt NaCl and as its only acid HCl. The pH of the interior of the erythrocyte has been taken as 7.3 and for simplicity all of its salts are represented as KCl. The concentrations given for the cell are merely roughly approximate values for arterial blood (see Henderson, 1928, page 196), since for our present purposes, where only the order of magnitude of the results is significant, a higher degree of accuracy would be meaningless.

As regards the external solution, we have two necessary conditions:

$$[H] \times [OH] = K_w = \text{approximately } 10^{-14}$$

and

$$[Cl] + [OH] = [H] + [Na].$$

Knowing [H] and [Na] which are here represented by x and y, respectively, the values of [Cl] and [OH] are immediately determined by the equations just given. In cases involving sums (but not products) of ionic concentrations in acid solutions, [OH] is usually so small that it may be neglected.

Under the conditions represented in Fig. 1, the directions and initial relative rates of ionic movement, for the two possible methods of pH change, would be determined by:

$$[H]_{solution} \times [Cl]_{solution} - [H]_{cell} \times [Cl]_{cell}$$

and

$$[OH]_{cell} \times [Cl]_{solution} - [OH]_{solution} \times [Cl]_{cell}$$

respectively. Substituting in these equations the various concentrations given in Fig. 1, we have:

$$x^2 + xy - 10^{-14} - 4 \times 10^{-9} \tag{1}$$

and

$$(2 \times 10^{-7})\left(x + y - \frac{10^{-14}}{x}\right) - \frac{8 \times 10^{-16}}{x}, \tag{2}$$

from which we may calculate, as has been done in Table IV, the relative tendencies for the erythrocyte to become more acid (or less

alkaline) in solutions of different degrees of acidity in the presence of an approximately physiological concentration (0.16 M) of NaCl and in its absence.

It is, of course, obvious that the figures in this table, taken singly, have no very great significance; and it is not even permissible at a given pH value to compare with each other the figures for hydrogen and for hydroxyl ions, since the actual rates of penetration of the cell by these ions will depend not merely on the mass law factors but upon the specific properties of the individual ions as well. But for each kind of ion separately over a series of different pH values, the figures have an important relative significance to which attention may now be directed.

It will be noted in Table IV that according to the hypothesis of

TABLE IV

Mass law effect with 0.16 M NaCl and isotonic saccharose solutions at different pH values, for movement across the cell membrane (a) of H· with Cl′ and (b) of OH′ in exchange for Cl′. Internal conditions as described in the text.

pH	0.16 M NaCl		Isotonic saccharose	
	H· with Cl′	OH′ in exchange for Cl′	H· with Cl′	OH′ in exchange for Cl′
7.0	12.00×10^{-9}	24.00×10^{-9}	-4.00×10^{-9}	-8.00×10^{-9}
6.0	15.60×10^{-8}	31.20×10^{-9}	-4.00×10^{-9}	-8.00×10^{-10}
5.0	15.96×10^{-7}	31.92×10^{-9}	-3.99×10^{-9}	-7.80×10^{-11}
4.0	16.01×10^{-6}	32.01×10^{-9}	6.00×10^{-9}	1.20×10^{-11}
3.0	16.10×10^{-5}	32.20×10^{-9}	9.96×10^{-7}	1.99×10^{-10}
2.0	17.00×10^{-4}	34.00×10^{-9}	1.00×10^{-4}	2.00×10^{-9}
1.0	26.00×10^{-3}	52.00×10^{-9}	1.00×10^{-2}	2.00×10^{-8}

permeability to hydrogen ions, the initial mass law factor, for the concentrations of acid actually employed in the experiments, should increase in the sodium chloride solution somewhat more rapidly than the concentration of hydrogen ions. In the saccharose solution, on the other hand, over the same range of concentrations the increase should be approximately proportional to the square of the hydrogen ion concentration. An examination of Tables II and III shows that in the salt solution the observed rate of penetration of the acid is only slightly affected by its own concentration, while in the saccharose solution it is roughly proportional to the first rather than to the second power of its concentration. Evidently the observed facts are in complete disagreement with the hypothesis of permeability to hydrogen ions.

On the other hand, both in the presence and the absence of NaCl

the rate of color change and of hemolysis are in good semi-quantitative agreement with the predicted mass law effects according to the hypothesis of permeability to hydroxyl ions. It is difficult to believe that this agreement is merely the result of chance. Admitting our ignorance of all but the probable beginning of the diffusion process, and making due allowance for the complicating effects of injury to the cells, especially in the more acid solutions, and the very rough nature of the calculations where so many simplifying assumptions have been made, it is nevertheless true that no other explanation of the facts has as yet been found which is at the same time so simple and so well in agreement with the other known properties of the erythrocyte.

The entire failure of color changes and of hemolysis to occur in sugar solutions at acid concentrations which readily bring them about in the presence of NaCl is a necessary consequence of the general theory of ionic exchanges. It will be noted in Table IV that for sugar solutions of pH 7.0, 6.0 and 5.0 the mass law factors for both mechanisms of ionic exchange have negative signs. That is to say, in such solutions the total movement of hydrogen or of hydroxyl ions, as the case may be, must be in the opposite direction from that found in the remaining solutions. In other words, erythrocytes in such solutions should theoretically become more alkaline rather than more acid.

This same problem may also be approached in a slightly different way by introducing the idea of a final equilibrium. Though it is obviously impossible, because of imperfect knowledge of the behavior of hemoglobin and of the erythrocyte at decidedly acid reactions, to calculate the final equilibrium conditions in cells exposed to any desired acid solution, it is nevertheless possible to determine what external solution would cause no internal pH change in normal cells. For the simplified erythrocyte already dealt with, either equation (1) or equation (2) (omitting quantities which are negligibly small) yields the following equation for equilibrium:

$$x^2 + xy = 4 \times 10^{-9}.$$

Solving for x, we have as the external hydrogen ion concentration in equilibrium with an internal pH value of 7.3:

$$x = \frac{\sqrt{y^2 + 1.6 \times 10^{-8}} - y}{2}. \qquad (3)$$

By means of this equation the pH values in Table V have been calculated.

It will be noted in this table that in the entire absence of salt the pH of equilibrium is 4.2, and therefore any solution less acid than this ought theoretically to cause the simplified erythrocyte to become more alkaline rather than more acid. For a concentration of NaCl of 0.0001 M, the critical pH is 4.5, and for one of 0.001, 5.4, etc. These figures, of course, cannot be expected to hold exactly in the case of actual erythrocytes where conditions are more complicated than those here considered. But the general principle itself, to which attention has already been directed by Netter (1928) and which has been put to a practical use by Bruch and Netter (1930), appears to be a sound one whose neglect has probably been responsible for considerable confusion in the past in experimental work with the erythrocyte.

TABLE V

External pH in equilibrium with an internal pH of 7.3 with various external concentrations of NaCl. Internal conditions as described in the text.

Concentration of NaCl (*mols. per liter*)	Equilibrium pH
—	4.2
0.0001	4.5
0.001	5.4
0.01	6.4
0.1	7.4

One further point about Tables II and III deserves mention. Not only is the color change of intact cells affected by the presence or absence of electrolytes, but a similar, though less marked, effect is observable in the case of hemolyzed blood. It is possible that we may here be dealing with a case similar to that reported by Adair, Barcroft, and Bock (1921), who found that even in blood hemolyzed by distilled water there is evidence that the cells, though invisible, may still be sufficiently well preserved to produce characteristic effects upon the dissociation curve of hemoglobin. It is not unreasonable, therefore, to expect that even in hemolyzed blood there might be some evidence of the same salt-acid effect that is found with intact cells. Whether this is a complete explanation of the observed facts, however, or whether some additional principle is involved cannot at present be stated with certainty.

SUMMARY

1. The penetration of acids into mammalian erythrocytes may be followed macroscopically by means of the color changes that occur when hemoglobin is converted into acid hematin.

2. The penetration of the acid precedes, rather than follows,

hemolysis. In certain cases, penetration may be observed without subsequent hemolysis.

3. Over a considerable pH range, both in the presence and in the absence of NaCl, the rate of acid penetration into the erythrocyte, as inferred from the time of color change, is in semi-quantitative agreement with that predicted for a system permeable to hydroxyl and not to hydrogen ions. There is an entire lack of agreement with the theoretical behavior of such a system when the permeability to the two ions is reversed. The simplest explanation of the observed facts is that the hydrogen ion, like other cations, is unable to enter the erythrocyte easily.

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